

University of Dundee

Identification of a proteasome-targeting arylsulfonamide with potential for the treatment of Chagas' disease

Lima, Marta; Tulloch, Lindsay; Corpas Lopez, Victoriano; Carvalho, Sandra; Wall, Richard; Milne, Rachel

Published in:
Antimicrobial Agents and Chemotherapy

DOI:
[10.1128/AAC.01535-21](https://doi.org/10.1128/AAC.01535-21)

Publication date:
2022

Document Version
Other version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Lima, M., Tulloch, L., Corpas Lopez, V., Carvalho, S., Wall, R., Milne, R., Rico Vidal, E., Patterson, S., Gilbert, I., Moniz, S., MacLean, L., Torrie, L., Morgillo, C., Horn, D., Zuccotto, F., & Wyllie, S. (2022). Identification of a proteasome-targeting arylsulfonamide with potential for the treatment of Chagas' disease. *Antimicrobial Agents and Chemotherapy*, 66(1), [e01535-21]. <https://doi.org/10.1128/AAC.01535-21>

General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Supplementary information

Table S1: Summary of primers used in RT-qPCR.

Primers	Sequences (5' - 3')
TcGAPDH_F	GTGCGGCTGCTGTCAACAT
TcGAPDH_R	AAAGACATGCCCCGTCAGCTT
TcMalic-Fw	ATAACATCTCCGCCAACGTC
TcMalic-Rv	AGTACACCGGCTTCCACATC
ProtB5qPCR-Fw	TGTGGGCTCAGGCTCTATCT
ProtB5qPCR-Rv	TTGCATGAAAAATGGAACGA

Table S2: Read number and fold coverage of whole genome sequencing analysis.

Table S3: Single nucleotide polymorphisms identified in open reading frames identified following of whole genome sequencing of compound **1**-resistant cell lines.

Table S4: RPKM and gene names of cosmid library 'hits' after selection with compound **1** (total region >5000 RPKM and >1 fragment).

Table S5: RPKM and gene names of cosmid library 'hits' after selection with compound **2** (total region >5000 RPKM and >1 fragment).

Table S6 – Collated EC₅₀ data for WT, resistant and transgenic *L. donovani* cell lines.

Cell line	Compound 1 EC ₅₀ values, μ M
	(fold change versus WT)
Wild-type	0.1 \pm 0.005 (-)
Compound 2 RES III*	26 \pm 4 (260)
cLdME ^{OE}	0.1 \pm 0.005 (=)

**L. donovani* cell line resistant to compound 2 bearing a G197S mutation in the β 5 subunit of the proteasome (12). All EC₅₀ values represent the weighted mean \pm standard deviation of at least three biological replicates ($n \geq 3$) with each biological replicate comprised of two technical replicates.

Table S7 - Collated EC₅₀ data for WT, resistant and transgenic *T. cruzi* cell lines in Vero cells.

Cell line	EC ₅₀ values, μ M (fold change versus WT)		
	Compound 1	GNF6702	Fexinidazole
Wild-type	1 \pm 0.2 (1)	0.2 \pm 0.03 (1)	4 \pm 0.8 (1)
RES 1	10 \pm 5 (8)	16 \pm 3 (85)	8 \pm 1 (2)
RES 5	>50 (>42)	3 \pm 0.4 (14)	4 \pm 0.7 (1)
β 5 ^{OE}	1 \pm 0.2 (1)	0.2 \pm 0.1 (1)	3 \pm 0.3 (1)
β 5 ^{OE} rescue R1	3 \pm 0.2 (2)	0.3* \pm 0.1 (2)	4 \pm 0.6 (1)
β 5 ^{D225N-OE}	12 \pm 1.4 (10)	> 5 (>25)*	3 \pm 0.6 (1)
β 4 ^{F24L/I29M}	>23 (>19)	> 1.5 (>8)	6 \pm 0.6 (2)
ME ^{OE}	0.8 \pm 0.07 (1)	0.05* \pm 0.01 (0.25)*	5 \pm 1 (1)

All data represents the weighted mean \pm standard deviation of three biological replicates with the exception of annotated values (*) which represent data from one biological replicate.

Protein ID	ΔT_m 1	p-value	ΔT_m 2	p-value	Protein name
C4B63_119g34	7.39	1.91E-06	3.89	0.001159	retrotransposon hot spot (RHS) protein
C4B63_11g96	-7.15	2.24E-07	-7.46	2.99E-05	protein kinase
C4B63_13g215	-5.52	0.009331	-3.59	0.138823	conserved hypothetical protein
C4B63_13g228	2.44	0.000813	3.17	0.138823	pre-mRNA-splicing factor ATP-dependent RNA helicase
C4B63_153g41	3.16	3.63E-06	2.00	0.075566	inositol 5-phosphatase 1(fragment)
C4B63_184g36	-4.95	0.041441	-4.16	0.013536	Vesicle-associated membrane protein 7
C4B63_188g44	-2.44	0.026678	-2.92	0.006095	conserved hypothetical protein
C4B63_218g24	2.67	0.000718	4.96	7.55E-07	Cullin family/Cullin protein neddylation domain containing protein
C4B63_22g269c	-2.86	0.05376	-3.81	9.12E-05	glutaredoxin
C4B63_26g233	-2.97	0.114299	-7.66	1.56E-05	mitochondrial DNA topoisomerase II
C4B63_28g106	8.60	4.28E-38	9.08	1.27E-15	malic enzyme
C4B63_297g18	2.10	0.041092	2.53	0.069381	conserved hypothetical protein
C4B63_2g455	4.59	5.05E-07	5.13	0.001469	Cytoplasmic dynein 2 heavy chain (DYNC2H1)
C4B63_2g691	-5.32	0.014042	-3.12	0.126048	30S Ribosomal protein S17
C4B63_328g5	-7.61	1.11E-08	-4.22	8.96E-06	conserved hypothetical protein
C4B63_41g242	-2.90	0.039256	-3.29	0.021793	conserved hypothetical protein
C4B63_42g60	-4.27	0.093074	-2.99	0.004584	amastin

C4B63_45g95	-2.64	0.005606	-5.82	5.46E-05	conserved hypothetical protein
C4B63_53g216	-2.98	0.095607	-4.15	0.00772	retrotransposon hot spot (RHS) protein
C4B63_61g142	2.25	0.024541	3.34	0.106747	conserved hypothetical protein

Table S8 - Top 20 hits identified by T_m analysis in biological replicate 1.

Protein ID	ΔT_m 1	p-value	ΔT_m 2	p-value	Protein name
C4B63_109g37	-9.66	8.7E-15	-12.47	6.09E-26	Gar1/Naf1 RNA binding region containing protein
C4B63_10g137	-2.43	0.005907	-3.25	0.141724	conserved hypothetical protein
C4B63_120g72	-7.25	1.15E-26	-10.99	1.81E-19	conserved hypothetical protein
C4B63_121g2	-7.14	5.15E-08	-9.76	2.04E-33	retrotransposon hot spot protein (RHS)
C4B63_12g292	-7.25	1.15E-26	-10.99	1.81E-19	conserved hypothetical protein
C4B63_14g113	-4.64	3.12E-10	-8.84	1.98E-09	conserved hypothetical protein
C4B63_158g46	5.23	0.003531	6.73	1.38E-09	retrotransposon hot spot protein (RHS)
C4B63_163g21	-8.95	1.13E-12	-9.86	3.86E-15	deoxyribose-phosphate aldolase
C4B63_16g205	-5.43	8.7E-15	-9.26	4.86E-13	retrotransposon hot spot (RHS) protein
C4B63_172g14	-6.42	1.81E-06	-11.54	9.52E-22	retrotransposon hot spot (RHS) protein
C4B63_17g1218c	7.00	0.001603	4.47	0.000788	conserved hypothetical protein
C4B63_20g223	-3.13	0.126456	-3.48	0.034656	SHQ1 protein
C4B63_20g307	4.53	3.23E-06	4.48	8.9E-09	conserved hypothetical protein
C4B63_226g19	5.23	0.003531	6.73	1.38E-09	retrotransposon hot spot protein (RHS)
C4B63_23g265	5.67	3.79E-10	2.89	6.55E-07	conserved hypothetical protein
C4B63_247g23	5.38	0.000609	4.09	4.46E-16	damage-specific DNA binding protein
C4B63_250g10	-6.31	3.02E-06	-7.27	2.86E-07	retrotransposon hot spot (RHS) protein

C4B63_28g106	5.60	2.08E-12	3.73	1.11E-06	malic enzyme
C4B63_2g415	3.27	4.37E-05	1.77	9.38E-06	conserved hypothetical protein
C4B63_31g213	2.57	0.003384	2.79	0.000783	conserved hypothetical protein

Table S9 - Top 20 hits identified by T_m analysis in biological replicate 2

Table S10 – Potency of an established ME inhibitor (ATR-073) against WT and ME^{OE} *T. cruzi* epimastigotes.

Cell line	ATR-073 EC ₅₀ values, μ M (fold change versus WT)
Wild-type	34 \pm 1
cTcME ^{OE}	32 \pm 2 (-)

All EC₅₀ values represent the weighted mean \pm standard deviation of at least three biological replicates (n \geq 3) with each biological replicate comprised of two technical replicates.

Supplementary figures

Figure S1 - Quantitative RT-PCR confirming overexpression of mutated and wild-type versions of the $\beta 5$ subunit of the proteasome in transgenic cell lines. The $\beta 5$ subunit bearing a D²²⁵N mutation was overexpressed in wild-type parasites while the unmutated subunit was overexpressed in compound **1**-resistant cell line RES 1.

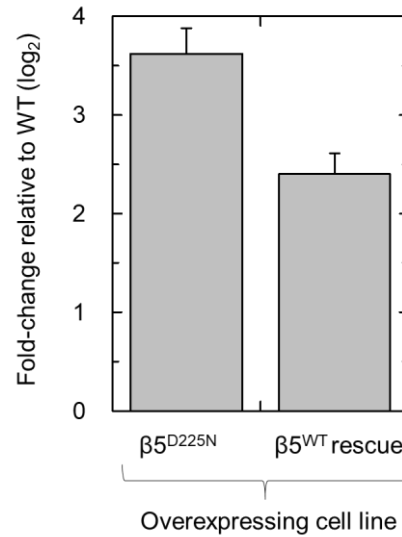


Figure S2 - Label-free proteomics quantitation. Relative protein levels in wild-type versus *cTcME*-overexpressing cell lines with *cTcME* indicated in red.

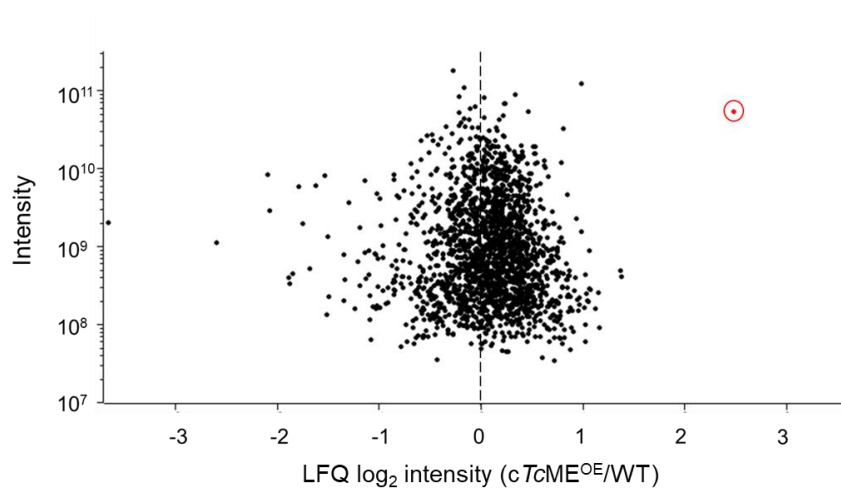


Figure S3 – Assessing the effect of ATR-073 on the *T. cruzi* proteasome. (A) Cell-free *T. cruzi* proteasome chymotrypsin-like activity concentration-response curves for ATR-073. At concentrations above 3.7 μM (indicated by a red line), ATR-073 began to interfere directly with the assay. Data are shown for 1 biological replicate ($n = 3$). The error bars represent SD. (B) EC_{50} values of 49 ± 29 , 49 ± 1 and 51 ± 18 μM were established for ATR-073 against WT (open circles), $\beta 4^{\text{F24L/I29M}}$ (blue circles) and compound **2**-Res V cells, respectively. These EC_{50} values are from one biological replicate, comprised of three technical replicates.

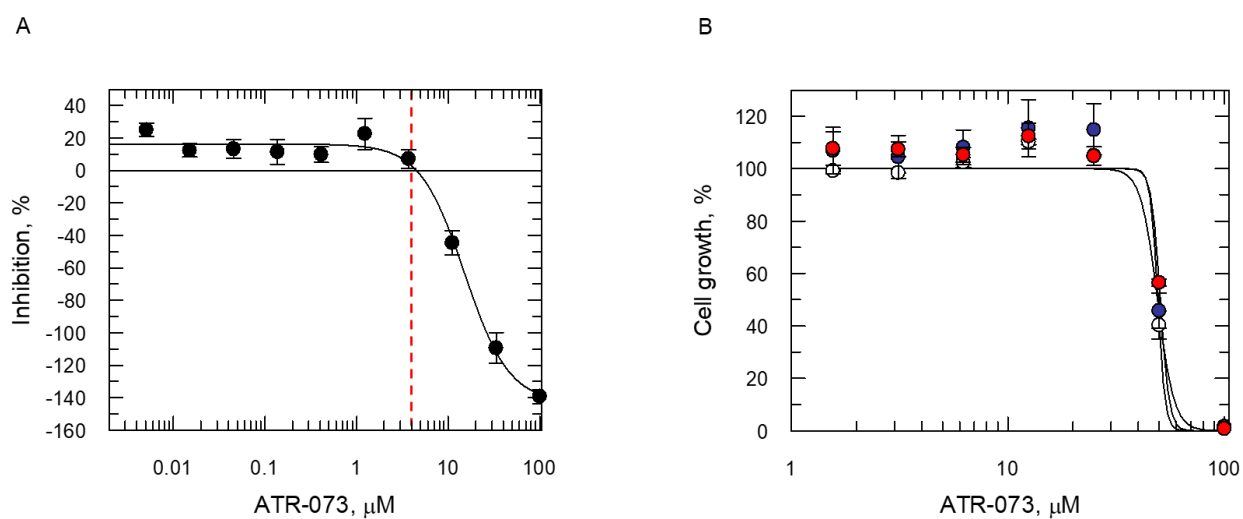
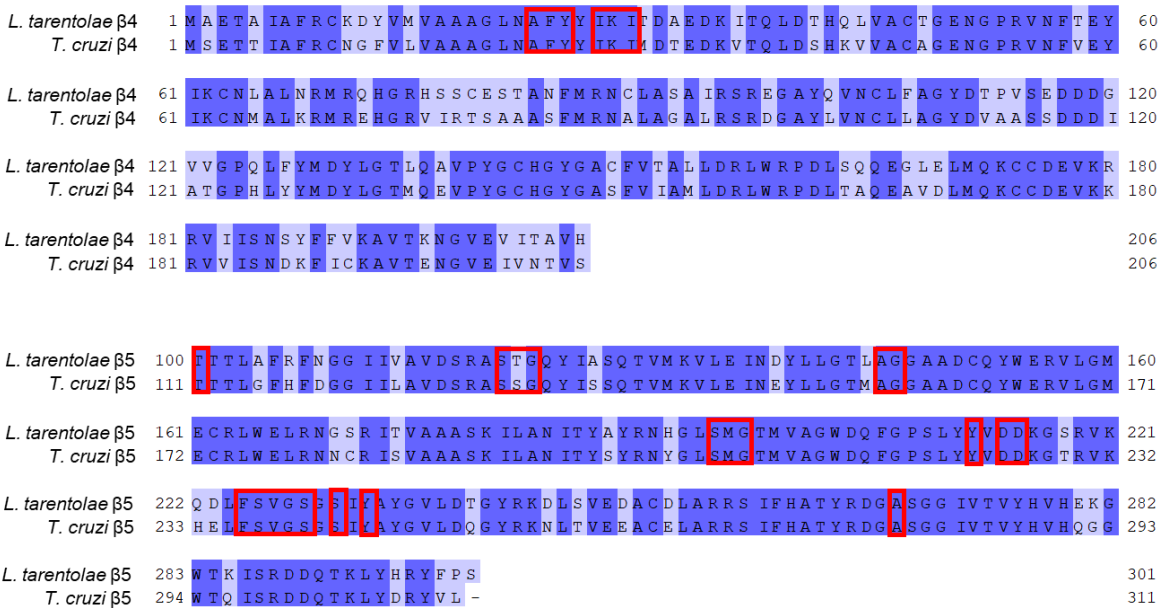


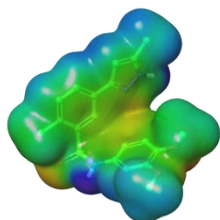
Figure S4 – Sequence alignment of $\beta 4$ and $\beta 5$ subunits of the proteasome. Amino acids within 5Å of the GSK3494245 binding site are identified in red boxes.



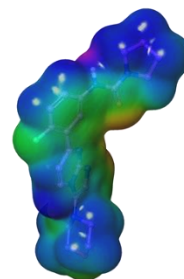
$\beta 4$ - $\beta 5$ subunit	<i>L. tarentolae</i>	<i>T. cruzi</i>
<i>L. tarentolae</i>	100	98
<i>T. cruzi</i>	98	100

Figure S5 – Electrostatic potential representation of compound **1** (A), GSK3494245 (B), and their respective localisation at the binding site colored by protein electrostatic potential (C,D).

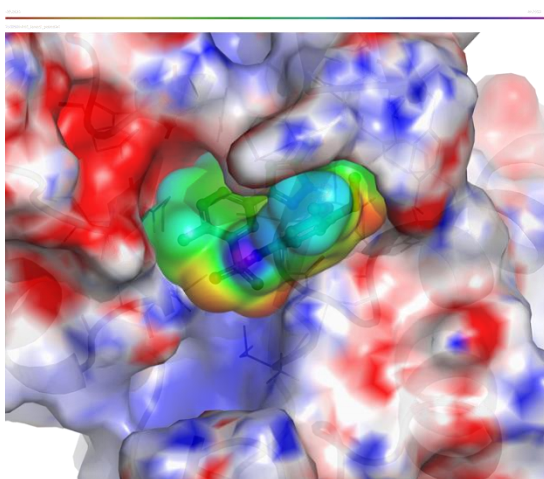
A



B



C



D

